

Data Evaluation Report on the Acute Inhalation Toxicity of Sulfuryl Fluoride in Bobwhite Quail (*Colinus virginianus*)

PMRA Submission Number {.....}

EPA MRID 48714701

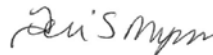
Data Requirement:	PMRA Data Code	{.....}
	EPA DP Barcode	405895
	OECD Data Point	{.....}
	EPA MRID	48942701
	EPA Guideline	OCSPP 870.1300, modified for bird species

Test Material:	Zythor	Purity:	100% (w:w)
Common name:	Sulfuryl fluoride		
Chemical name:	IUPAC: sulfuryl fluoride		
	CAS name: sulfuryl fluoride		
	CAS No.: 2699-79-8		
	Synonyms: none specified		

Primary Reviewer: Christie E. Padova
Staff Scientist, CSS-Dynamac Corporation

Signature: 
Date: 06/18/14

Secondary Reviewer: Teri S. Myers
Senior Scientist, CMD Smith

Signature: 
Date: 07/16/14

Primary Reviewer: {.....}
{EPA/OECD/PMRA}

Date: {.....}

Secondary Reviewer(s): {.....}
{EPA/OECD/PMRA}

Date: {.....}

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	078003	

Date Evaluation Completed: {dd-mm-yyyy}

Citation: Weinberg, J.T. 2012. Acute Inhalation Toxicity Study of Sulfuryl Fluoride in Northern Bobwhite Quail. Unpublished study performed by WIL Research, Ashland, OH. Laboratory Project ID: WIL-842001. Study sponsored by Ensystex II, Inc., Fayetteville, NC and Drexel Chemical Company, Memphis, TN. Study initiated January 23, 2012 and completed September 14, 2012.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute inhalation effects of a pesticide on avian species. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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EXECUTIVE SUMMARY: In an acute inhalation toxicity study, 32- to 34-week old Northern bobwhite quail (five per sex, 195 to 247 g) were exposed by whole-body inhalation to sulfuryl fluoride for 4 hours at average exposure chamber concentrations of 0 (control), 825, 1159, 1423, and 1727 ppm. The animals were observed for up to 14 days post-exposure then subjected to gross pathological examination. The acute inhalation LC₅₀ (with 95% C.I.) for was 1620 (1470 to 2030) ppm for combined sexes; the study author additionally identified sex-specific LC₅₀s as 1569 (1424 to 1729) ppm for males, and 1728 (1377 to 2168) ppm for females.

Cumulative mortality was 0/10, 0/10, 0/0, 3/10 and 6/10 animals for the 0, 825, 1159, 1423 and 1727 ppm groups, respectively. All mortality at the 1423 ppm level occurred by Day 1. Mortality at the 1727 ppm level occurred either during exposure (4/10) or by Day 1. During the exposure period, effects such as ataxia, excessive head shaking, hyperactivity, wiping head on cage, prostration, intermittent tremors, convulsions, gasping, labored respiration, and partial eye closures were observed in birds from the 1423 and 1727 ppm treatment levels. Similar effects were observed in birds from all levels immediately following exposure. In addition, hypoactivity, body cool (to touch), lacrimation, rales, decreased respiration, complete eye closure and/or emesis were also observed immediately following exposure in birds from the ≥ 1159 ppm levels. Surviving animals in the 825, 1159 and 1423 ppm levels were considered normal by Days 5, 3, and 14, respectively. Some clinical effects persisted through the duration of the observation period at the 1727 ppm level.

During the 0- to 7- and 0- to 14-day intervals, there was a significant decrease in body weight change compared to the control group for males in all test substance groups ($p \leq 0.01$). For the females, there was a significant decrease in body weight change compared to the control group during the 0- to 7-day interval at the 1423 and 1727 ppm groups and during the 0- to 14-day interval for the 1727 ppm group ($p \leq 0.01$). No apparent treatment-related effect on food consumption was observed.

There were no treatment-related findings upon gross necropsy of decedent animals. In birds examined following the 14-day observation period, observations noted at necropsy were white areas of the liver in one male in the 825 ppm group, dark red discoloration of the thyroid glands for one male in the 1159 ppm group, and small thyroid glands in one female from the 1727 ppm group.

This toxicity study is classified as **scientifically sound/unsound and does/does not** satisfy the guideline requirement for an acute inhalation toxicity study with Northern bobwhite quail.

Results Synopsis:

Study author-reported results

LC ₅₀ (male): 1569 ppm	95% C.I.: 1424 to 1729 ppm
Probit slope: 1.12	95% C.I.: 1.07 to 1.17

LC ₅₀ (female): 1728 ppm	95% C.I.: 1377 to 2168 ppm
Probit slope: 1.30	95% C.I.: 1.02 to 1.64

Reviewer-reported results

LC ₅₀ (combined): 1620 ppm	95% C.I.: 1470 to 2030 ppm
Probit slope: 13.3	95% C.I.: 3.89 to 22.7

Endpoint(s) Affected: mortality, clinical signs of toxicity, body weight changes

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I. MATERIALS AND METHODS

GUIDELINE(S) FOLLOWED:

The study protocol was based upon procedures outlined in the U.S. EPA OPPTS Guideline 870.1300 (1998), U.S. EPA OPPTS Guideline 850.1200 (1996) and OECD Guideline No. 403.

General guideline deviations included:

- Food weight was inadvertently not recorded on study days 7 and 14 for the 1423 ppm group and on study day 14 for the 825 ppm group.
- The analytical LOD and/or LOQ were not reported.

COMPLIANCE:

Signed and dated Data Confidentiality, GLP, and Quality Assurance statements were provided. The study was conducted in compliance with EPA GLP Standard (40 CFR Part 160) and OECD Principles of GLP, with the following exceptions: basal diet analysis, and range-finding data reporting.

A. MATERIALS:

1. Test Material: Zythor (sulfuryl fluoride)

Description: Clear colorless gas

Lot/Batch No.: LU11001 (exp. 03/20/14)

Purity: 100%

Stability of compound under test conditions: Minimal analytical variation was observed during the exposure periods, with reviewer-calculated coefficients of variation (CV) of 1.4 to 3.0%.

Storage Conditions: Room temperature

Physicochemical properties of sulfuryl fluoride.

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	11,552 mm Hg at 20°C	Vapor density: 4.3 g/L at 20°C [MRID 48714701]
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

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2. Test Organism:

Species: Northern bobwhite quail (*Colinus virginianus*)

Age at exposure: 32 to 34 weeks old

Weight at exposure: 195 to 247 g males, 197 to 243 g females

Source: Buckeye Gamebirds LLC, Defiance, OH

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: Three 4-hour range-finding exposures were performed with two birds per sex per level at analyzed exposure concentrations of 504, 1025 and 1596 ppm. Results were not reported.

b. Definitive study:

Table 1: Experimental Parameters

Parameter	Details	Remarks
<u>Acclimation</u> Period:	≥14 days	The basal diet was analyzed for nutritional content and the presence of heavy metals and pesticides, and the water was analyzed for contaminants. No contaminants were present in the feed or water at concentrations sufficient to interfere with the objectives of the study.
Feeding:	Game Bird Flight Conditioner® 5435 (Purina Mills) and municipal water were provided <i>ad libitum</i> , except during exposure.	
Health: (any mortality observed)	During acclimation, birds were observed twice daily for mortality and moribundity.	

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Parameter	Details	Remarks
Pen size and construction materials	Upon arrival, all animals were group-housed (≤ 5 quail per sex per pen) in suspended wire-mesh cages. The birds were transported to the exposure room in a wire-mesh battery containing separate cages, and following exposure, were returned to their home cages for a 14-day observation period.	
<u>Number of birds per treatment</u> control: treated:	10 (5 per sex) 10 (5 per sex) per level	
<u>Environmental conditions</u> Temperature: Relative humidity: Photoperiod:	20.8 to 22.1°C 36.1 to 55.2% 8 hours light/16 hours dark	≥ 10 air changes per hour (average) Music in the form of nature/bird sounds was played in the housing room.
Exposure duration Test duration	Single, 4 hours 14-day post-exposure observation period	The animals were removed from the exposure chamber after the t_{99} clearance time (see below).
Where food and water withheld during exposure?	Yes	
<u>Exposure levels</u> target: measured:	0 (control) 800, 1100, 1400 and 1700 ppm 0 (control), 825, 1159, 1423 and 1727 ppm	Actual exposure concentrations were determined at <i>ca.</i> 30-minute intervals using a gas chromatograph/thermal conductivity detector (GC/TCD). The analytical LOD and/or LOQ were not reported. Minimum analytical variation was observed during the 4-hour exposure periods, with reviewer-calculated coefficients of variation of 1.4 to 3.0% for all levels.

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Parameter	Details	Remarks
<u>Exposure Conditions:</u> Mass median aerodynamic diameter (MMAD): Time to equilibration: Oxygen content: Temperature: Humidity: Air changes:	N/A $t_{99} = 20$ minutes 20.9% 21°C – mean control 23 to 24°C – mean treatment levels 38 to 49% - mean all levels ≥ 12 per hour	Chamber temperature, relative humidity, ventilation rate, and negative pressure were recorded every 35 minutes during exposure. Oxygen content was determined during the pre-exposure method development phase, and also measured in the definitive test at the target 800, 1100 and 1400 ppm levels. As sulfuryl fluoride is a true gas, calculation of the MMAD was not applicable.
Test Atmosphere Generation	The (gaseous) test material was metered via a 2-stage regulator and needle valve into the inlet of the exposure chamber. To obtain sufficient pressure within the cylinder for the 825 and 1159 ppm groups, a heat blanket was used to heat the cylinder (to <i>ca.</i> 30°C). In addition, for all exposures, the regulator and tubing were heated (<i>ca.</i> 60°C) using heat tape. The test material was mixed with supply air to achieve the desired concentrations.	
<u>Reference chemical, if used</u> name: concentrations tested:	None tested	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
<u>Parameters measured</u>	- Mortality - Clinical signs of toxicity - Body weight - Food consumption - Necropsy	

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Criteria	Details	Remarks
Observation intervals	Birds visible through the chamber windows were observed for mortality and clinical signs of toxicity hourly throughout exposure, immediately following exposure, and at least once daily thereafter for 14 days. Body weights were determined on Days 0 (just prior to exposure), 7, and 14. Food consumption was recorded weekly.	
Groups on which necropsies were performed	The major organ systems of the cranial, thoracic and abdominal cavities were grossly examined for all animals at the time of death.	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Cumulative mortality was 0/10, 0/10, 0/0, 3/10 and 6/10 animals for the 0, 825, 1159, 1423 and 1727 ppm groups, respectively. A single male (no. 3100) from the 825 ppm group was euthanized *in extremis* due to a suspected cage-related accident resulting in a fractured leg bone. As there were no other deaths at the 825 ppm level and no mortality at the 1159 ppm level, this single male was not included in the LC₅₀ calculation. All mortality at the 1423 ppm level occurred by Day 1. Mortality at the 1727 ppm level occurred either during exposure (4/10) or by Day 1. The acute inhalation LC₅₀ value (with 95% C.I.) for combined sexes was reported to be 1622 (1458 to 1805) ppm with a slope of 1.19 (1.10 to 1.28).

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Table 3. Effect of Sulfuryl Fluoride on Mortality of Northern Bobwhite Quail.

Measured Concentrations (ppm)	No. of Birds	Cumulative Mortality										
		During Exposure		Days 0-1		Days 2-6		Days 7-14		Total		
		M	F	M	F	M	F	M	F	M	F	C
Control	10/sex	0	0	0	0	0	0	0	0	0	0	0
825	5/sex	0	0	0	0	0	0	0	0	0	0	0
1159	5/sex	0	0	0	0	0	0	0	0	0	0	0
1423	5/sex	0	0	1	2	0	0	0	0	1	2	3
1727	5/sex	3	1	1	1	0	0	0	0	4	2	6
LC ₅₀ (with 95% C.I.) ^(a)	Male: 1569 (1424 to 1729) ppm Female: 1728 (1377 to 2168) ppm Combined: 1622 (1458 to 1805) ppm											

^(a) Animal no. 3100 (825 ppm level) was not included in LC₅₀ calculation.

B. SUBLETHAL TOXICITY ENDPOINTS:

No clinical signs of toxicity were observed at the control level at any time during the study. During the exposure period, effects such as ataxia, excessive head shaking, hyperactivity, wiping head on cage, prostration, intermittent tremors, convulsions, gasping, labored respiration, and partial eye closures were observed in birds from the 1423 and 1727 ppm treatment levels. Similar effects were observed in birds from all levels immediately following exposure. In addition, hypoactivity, body cool (to touch), lacrimation, rales, decreased respiration, complete eye closure and/or emesis were also observed immediately following exposure in birds from the ≥ 1159 ppm levels. During the 14-day post-exposure period, female no. 3165 (825 ppm level) showed clinical observations including unkempt appearance, thinness, red material on the left leg, laceration of the left leg, impaired use of left leg, and feather loss of dorsal trunk and rump. It was suspected that the effects were the result of aggression by the cage mates. This single bird also exhibited a large body weight loss. Surviving animals in the 825, 1159 and 1423 ppm levels were considered normal by Days 5, 3, and 14, respectively. Some clinical effects persisted through the duration of the observation period at the 1727 ppm level.

In male birds, treatment-related effects on body weight changes were observed at all levels. Between 0 and 7 days, male body weight changes averaged -2, -14, -11, -15 and -16 g for the 0 (control), 825, 1159, 1423 and 1727 ppm treatment levels, respectively. As there was only one surviving male in the 1727 ppm group, statistical analysis could not be performed at this level; however, differences were statistically-significant ($p \leq 0.01$) compared to the control at the 825, 1159 and 1423 ppm levels and the reduction observed at the 1727 ppm level was considered to be treatment-related. Between 0 and 14 days, male body weight changes averaged 0, -10, -12, -9 and -10 g for the 0, 825, 1159, 1423 and 1727 ppm levels, respectively. Differences were statistically-significant compared to the control at the $p \leq 0.01$ level for the 825 and 1159 ppm groups and at $p \leq 0.05$ significance at the 1423 ppm level. For evaluation of body weight changes in females, animal no. 3165 (female from the 825 ppm level) was excluded from statistical analysis of body weight due to the large body weight decrement resulting from cage-mate aggression as described above. Treatment-related effects on body weight changes were observed at the two highest treatment levels in female birds. Between 0 and 7 days, female body weight changes averaged -2, -8, -4, -16 and -19 g for the 0 (control), 825, 1159, 1423 and 1727 ppm treatment levels, respectively; differences were statistically-significant compared to the control at the 1423 and 1727 ppm levels ($p \leq 0.01$). Between 0 and 14 days, female body weight changes averaged -1, -4, -8, -10 and -14 ppm; the difference was statistically-significant compared to the control at the 1727 ppm level ($p \leq 0.01$).

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Table 4. Effect of Sulfuryl Fluoride on the Body Weight of Northern Bobwhite Quail.

Mean Body Weights \pm SD, g (and Changes from Day 0, g)						
Measured Concentrations (ppm)	Males			Females		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control	220 \pm 7.9	218 \pm 7.7 (-2 \pm 1.9)	220 \pm 6.9 (0 \pm 2.4)	219 \pm 7.0	217 \pm 4.1 (-2 \pm 3.4)	218 \pm 4.0 (-1 \pm 4.1)
825	216 \pm 18.5	207 \pm 11.2 (-14 \pm 7.3)**	211 \pm 12.8 (-10 \pm 5.2)**	202 \pm 5.4	195 \pm 5.0* (-8 \pm 1.3)	198 \pm 5.8 (-4 \pm 2.4)
1159	216 \pm 12.9	205 \pm 11.5 (-11 \pm 2.3)**	204 \pm 11.9 (-12 \pm 3.8)**	210 \pm 11.8	205 \pm 11.0 (-4 \pm 2.7)	202 \pm 11.1 (-8 \pm 2.2)
1423	220 \pm 12.0	209 \pm 7.0 (-15 \pm 3.3)**	215 \pm 6.7 (-9 \pm 4.3)*	214 \pm 10.9	194 \pm 21.0* (-16 \pm 9.9)**	199 \pm 21.5 (-10 \pm 9.1)
1727	214 \pm 11.2	201 \pm 0.0 (-16 \pm 0.0) ^(a)	207 \pm 0.0 (-10 \pm 0.0) ^(a)	228 \pm 11.8	203 \pm 7.0 (-19 \pm 6.4)**	209 \pm 8.1 (-14 \pm 5.1)**

*Statistically-significant compared to the control at $p \leq 0.05$.

** Statistically-significant compared to the control at $p \leq 0.01$.

^(a) N=1; statistical analysis was not conducted.

No treatment-related effect on feed consumption was observed when the control and test substance-treated groups were compared. Weekly food consumption averaged 11 to 20 g/animal/day for all levels.

Table 5. Effect of Sulfuryl Fluoride on the Food Consumption of Northern Bobwhite Quail.

Mean Weekly Food Consumption, g/animal/day				
Measured Concentrations (ppm)	Males		Females	
	Days 0 to 7	Days 7 to 14	Days 0 to 7	Days 7 to 14
Control	20	15	16	18
825	11	Not determined ^(a)	12	Not determined
1159	12	17	12	15
1423	Not determined	Not determined	Not determined	Not determined
1727	15	16	11	13

*Statistically-significant compared to the control at $p \leq 0.05$.

** Statistically-significant compared to the control at $p \leq 0.01$.

^(a) Food weight was inadvertently not recorded on study days 7 and 14 for the 1423 ppm group and on study day 14 for the 825 ppm group.

There were no treatment-related findings upon gross necropsy of decedent animals. In birds examined following the 14-day observation period, observations noted at necropsy were white areas of the liver in one male in the 825 ppm group, dark red discoloration of the thyroid glands for one male in the 1159 ppm group, and small thyroid glands in one female from the 1727 ppm group. No other macroscopic findings were noted.

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C. REPORTED STATISTICS:

Statistical method: The acute 4-hour LC₅₀ values for males and females (separately) were determined using the method of Litchfield and Wilcoxon (1949). Dunnett's test was used to identify treatment levels that were statistically-significantly different from the control group at the $p \leq 0.01$ and $p \leq 0.05$ levels. Results were provided in terms of measured ppm.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer verified the LC₅₀ for combined sexes using the 850.2100 test design and the linear regression model via CETIS v. 1.8.7.12 with backend settings implemented by EFED on 03/25/14. Day 0 to 14 body weight change and average standard errors for this time interval were entered into the test code for this data set, but these endpoints were not statistically analyzed because there is no current provision for them in the analytical test design. Toxicity values were based on the measured concentrations.

LC₅₀ (combined): 1620 ppm
Probit slope: 13.3

95% C.I.: 1470 to 2030 ppm
95% C.I.: 3.89 to 22.7

E. STUDY DEFICIENCIES:

There were no significant deficiencies from U.S. EPA OCSPP Guideline No. 870.1300 affecting the scientific soundness or acceptability of this study. Based upon potential exposure following field application, the study was conducted as a whole-body exposure with Northern bobwhite quail, considered to be a more sensitive bird species (presumably in addition to a mammalian species). The methods were modified as necessary to accommodate a bird species.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions generally agreed with the study author's; the reviewer used EFED-approved statistical methods to verify the 14-day combined sex LC₅₀, so the reviewer's results for this endpoint are reported in the Executive Summary and Conclusions sections.

The homogeneity of the exposure concentrations was assessed during the method development phase of the study. Samples were collected from two test locations in the middle chamber position and a reference location. The measured concentration was calculated as a percent difference for each position from the reference location. All mean measurements were within 2.6% from the reference, indicating that the homogeneity of the chamber atmosphere was adequate for the purpose of the study.

Experimental study dates were January 25 to May 23, 2012.

G. CONCLUSIONS:

This study is **scientifically sound/unsound and is thus acceptable/unacceptable**. Based upon the results of the study, the acute inhalation LC₅₀ was 1569 and 1728 ppm for male and female Northern bobwhite quail, respectively (study author-reported results). For combined sexes (reviewer-reported results), the acute inhalation LC₅₀ was 1620 ppm, with 95% C.I. of 1470 to 2030 ppm. Clinical signs of toxicity were observed at all levels; effects included hyperactivity, ataxia, excessive head shaking, labored respiration, prostration, gasping, lacrimation, tremors, convulsions, partial to complete closure of the eye(s), hypoactivity, rales, decreased respiration, emesis containing clear material, thinness, and unkempt appearance. Treatment-related effects on body weight changes were observed at all levels for male birds and at the two highest treatment levels (1423 and 1727 ppm) in female birds. No apparent effect on food consumption was indicated in surviving birds. Macroscopic findings upon necropsy of surviving birds included white areas of the liver in one male in

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the 825 ppm group, dark red discoloration of the thyroid glands for one male in the 1159 ppm group, and small thyroid glands in one female from the 1727 ppm group.

Study author-reported results

LC₅₀ (male): 1569 ppm

95% C.I.: 1424 to 1729 ppm

Probit slope: 1.12

95% C.I.: 1.07 to 1.17

LC₅₀ (female): 1728 ppm

95% C.I.: 1377 to 2168 ppm

Probit slope: 1.30

95% C.I.: 1.02 to 1.64

Reviewer-reported results

LC₅₀ (combined): 1620 ppm

95% C.I.: 1470 to 2030 ppm

Probit slope: 13.3

95% C.I.: 3.89 to 22.7

Endpoint(s) Affected: mortality, clinical signs of toxicity, body weight changes

III. REFERENCES:

Litchfield, J.T., and F.A. Wilcoxon. 1949. A Simplified Method of Evaluating Dose-Effect Experiments. *Journal of Experimental Therapeutics* 96:99-113.

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APPENDIX I. REVIEWER'S BODY WEIGHT CHANGE (GRAMS) CALCULATIONS:

	Day 0		Day 7		Day 14		Day 0-14 males	Day 0-14 females
	Males	Females	Males	Females	Males	Females		
Control	220	219	218	217	220	218	0	-1
STd	7.9	7	7.7	4.1	6.9	4		
n	5	5	5	5	5	5		
Stderr	1.58	1.4	1.54	0.82	1.38	0.8	1.48	1.1
825 ppm	216	202	207	187	211	191	-5	-11
STd	18.5	4.8	11.2	17.4	12.8	16.4		
n	5	5	5	5	5	5		
STderr	3.7	0.96	2.24	3.48	2.56	3.28	3.13	2.12
1159 ppm	216	210	205	205	204	202	-12	-8
STd	12.9	11.8	11.1	11	11.9	11.1		
n	5	5	5	5	5	5		
STderr	2.58	2.36	2.22	2.2	2.38	2.22	2.48	2.29
1423 ppm	220	214	209	194	215	199	-5	-15
STd	12	10.9	7	21	6.7	21.5		
n	3	5	4	3	4	3		
STderr	4	2.18	1.75	7	1.675	7.166667	2.84	4.673333
1727 ppm	214	228	201	203	207	209	-7	-19
STd	11.2	11.8	0	7	0	8.1		
n	5	5	1	3	1	3		
STderr	2.24	2.36	0	2.333333	0	2.7	1.12	2.53